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Original Paper

Involvement of a tobacco leucine-rich repeat-extensin in cell morphogenesis

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Abstract The unique mutant *nolac-K4* (non-organogenic callus with loosely attached cells), which was generated by T-DNA transformation using leaf-disk cultures of haploid *Nicotiana plumbaginifolia*, has lost the ability to form adventitious shoots and also shows decreased intercellular attachment. The gene tagged with the T-DNA in this line, named *NpLRX1* (LRR-EXTENSIN 1), is a novel tobacco gene that encodes a cell-wall protein containing chimeric leucine-rich repeat and extensin domains. The gene is highly similar to the *Arabidopsis LRX* genes, and phylogenetic analysis places it in the vegetative *LRX* clade. *NpLRX1* is expressed ubiquitously in vegetative tissues, strongly in the leaf and root vascular bundles, and in emerging lateral roots and root tips. Tobacco leaf disks transformed with an *NpLRX1*-RNAi construct displayed aberrant adventitious buds and disorganized cell morphogenesis with large intercellular spaces. The shapes of *NpLRX1*-RNAi-transformed BY-2 cells were irregular, and the cells showed disorganized cortical microtubules. These results suggest that *NpLRX1*, the lack of which may be responsible for the *nolac-K4* mutation, is a new tobacco *LRX* gene that has regulatory roles in cell morphogenesis that are essential for plant tissue development.

Key words: Cell morphogenesis, cell wall, LRR-extensin, *nolac* mutant, *Nicotiana*.

Spatially and temporally controlled cell growth and intercellular communication are indispensable for tissue organization, making these processes critical for normal development and morphogenesis in every multicellular organism. Interactions through the extracellular matrix play an important role in controlling many developmental decisions. In plants, the extracellular matrix that encompasses each cell, the cell wall, forms a single continuous matrix throughout the body of the plant. Cell walls are highly complex and heterogeneous, and have diverse components that play wide-ranging and complex roles in the regulation of cell functions. The components include polysaccharides and proteins such as arabinogalactan proteins (AGPs: Hengel et al. 2001) and hydroxyproline-rich glycoproteins (Wu et al. 2001). The genes that encode many cell wall proteins are expressed in cell-specific developmentally regulated manners and are essential for normal development. For example, the root-hair-specific chimeric protein *AtLRX1*, which contains a leucine-rich repeat (LRR) domain, a motif that mediates specific protein-protein interaction, and an extensin domain, a motif related to a hydroxyproline-rich cell wall protein, is required for the normal polarized growth of Arabidopsis root hairs (Baumberger et al. 2001).

In this study, we report the molecular and functional characterization of the tobacco LRR-EXTENSIN gene (*NpLRX1*: LRR-EXTENSIN 1). This gene was identified from the *nolac-K4* mutant, which is defective in intercellular attachment. The *nolac* mutants were produced by T-DNA transformation (Iwai et al. 2001), which involves the *in vitro* culture of leaf disks of haploid *N. plumbaginifolia* (Marion-Poll et al. 1993). Haploid *N. plumbaginifolia* plants are ideal for generating and studying such mutants because mutations have a direct effect on the phenotype and the cells can grow as unorganized callus. In the more frequently used system for plant mutant production, generating mutants in *Arabidopsis* plants, the mutations that cause defects in the essential cell-wall components sometimes result in embryonic-lethal phenotypes. Our system permits the *in-vitro* culture of these lethal mutants as non-organogenic

Abbreviations: GFP, green fluorescent protein; GUS, β-glucuronidase; LRR, Leucine-rich repeat; LRX, Leucine-rich repeat-extensin; *nolac*, non-organogenic callus with loosely attached cells; RNAi, RNA interference; RT-PCR, reverse transcription-polymerase chain reaction; TAIL-PCR, thermal asymmetric interlaced-polymerase chain reaction.

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This article can be found at http://www.jspcmb.jp/

callus, enabling the subsequent analysis of the characteristics of the mutant (Iwai et al. 2002).

However, even in *in-vitro* callus culture, the cells of the *nolac-K4* line were difficult to maintain because of the weak growth of this line, similar to a number of other *nolac* mutants. Consequently, the *nolac-K4* line perished before the mutant characteristics were sufficiently analyzed. Nevertheless, we identified a gene in the T-DNA-flanked DNA of this line that encodes *NpLRX1*. This finding allowed us to generate transformed tobacco plants (*N. plumbaginifolia* and *N. tabacu*m) and BY-2 cells in which the expression of the endogenous *NpLRX1* or *NtLRX1* was suppressed by RNAi, and we used these lines to investigate the phenotypes of tobacco *LRX1*-deficient cells. The transformants showed disorganized cell morphology and decreased cell adhesion ability. These results suggest that *NpLRX1* has regulatory roles in cell morphogenesis that are essential for normal tissue development.

Materials and methods

Plant materials

Haploid *N. plumbaginifolia* plants were a gift from the Institut National de la Recherche Agronomique (INRA), Centre de Versailles, France. *N. plumbaginifolia* and *N. tabacum* plants were grown on Murashige and Skoog (MS) medium prepared with 1/2-strength macronutrients, 30 g l^{-1} sucrose, and 0.7% agar. All plants were grown at 25°C with a 16-h light/8-h dark cycle (white light at 50 μmol photons m^{-2} s^{-1}).

Tobacco BY-2 cells and BY-GT 16 cells (Kumagai et al. 2001) were grown in 95 ml of a modified Linsmaier and Skoog (LS) medium (Linsmaier and Skoog 1965) at 25°C in darkness with agitation on a rotary shaker at 120 rpm. The cells were freshly subcultured every week.

Generation and screening of nolac mutants

The transformation vector used to generate the *nolac* mutant was the pBI121 binary T-DNA vector (Clontech), which contains a kanamycin resistance marker for the selection of transformed plants. The vectors were introduced into *Agrobacterium tumefaciens* strain LBA4404.

Leaf disks of haploid *N. plumbaginifolia* were transformed using a modified version of the leaf-disk method (Horsch et al. 1985). Every two weeks, the leaf disks were transferred and cultured on MS medium containing 0.8% agar, 1 mg l^{-1} benzyladenine for shoot induction, 100 mg l^{-1} kanamycin to select transgenic cells, and 500 mg l^{-1} Claforan to remove *Agrobacteriu*. By four weeks of culture, numerous calli had regenerated. We selected paste-like callus that did not form adventitious shoots and showed weak intercellular attachments. We confirmed the characteristics of the paste-like callus by touching it with tweezers to test the hardness of the callus.

Isolation of the NpLRX1 and NtLRX1 genes

To amplify the fragment containing the region flanking the T-DNA insertion, we performed TAIL-PCR using genomic DNA extracted from *nolac-K4* callus as the template (Liu and Whittier 1995). A fragment of *NpLRX1* containing a putative open reading frame was amplified from *N. plumbaginifolia* cDNA using the primers NpLRX1-XhoS (5′-ctcgagATAG TAGCAATGCGGCCTCCT-3′) and LREX-L3 (5′-GGAAAA TTAAAGATAATGAACTGAGGCTAA-3′). The underlined sequence is a non-native *XhoI* restriction site, which was included in the primer for subcloning purposes. The *NtLRX1* fragment was amplified from *N. tabacum* genomic DNA by PCR with the above primers. Alignment of the predicted amino acid sequences and generation of the phylogenetic tree were performed using CLUSTAL X (Genetics Computer Group).

Expression analysis

Total RNA was extracted from various organs of transgenic and non-transformed plants using an RNAqueous kit (Ambion). For expression analysis by RT-PCR, first-strand cDNA was synthesized using a QuantiTect Reverse Transcription Kit (Qiagen) and 1 μg of total RNA. A 1-μl aliquot of the products of this reaction was used as a template in a 50-μl PCR reaction employing Promega Taq (Promega). PCR was carried out in the presence of gene-specific primers. To monitor whether equal amounts of cDNA were synthesized, a cDNA fragment of the constitutively expressed actin gene (accession number AB158612) was amplified simultaneously in 25 or 30 cycles. The primer sequences and predicted amplicon sizes are as follows: *NtLRX1* [196 bp; NtLRX1-RT-S, 5′-AGATCTGCT AAAGAATGCTCTTCTGAT-3′ and NtLRX1-RT-AS, 5′- CA ATTGGTACAATAGGTTTTGTAGGA-3′]; and *Actin* [665 bp; Actin-RT-S, 5′TCTGGCATCATACCTTTTACAATGAG-3′ and Actin-RT-AS, 5′- AAACATTGTTGTTCCACCACTAAGG-3′].

Construction of binary vectors and transformation

A fragment of the *NpLRX1* promoter (between −1307 and −132 bp from the translation start site), with *Eco*RI and *Bam*HI sites added for subcloning purposes, was amplified using the primers NpLRX1-PG-U (5′-cccaagcttgggAATTTTCTCGCC TGATCAAGCGGGA-3′) and NpLRX1-PG-L (5′-cggatccc GGTTTGTAAGTATTGAGAAGATGGGC-3′). The amplified fragment was fused between the *Hin*dIII and *Bam*HI sites of the pBI101 vector (Clontech).

To generate *NpLRX1*-RNAi transgenic plants, the region of *NpLRX1* between 1367 and 1551 bp was amplified using the primers NpLRX1-RNAi-S (5′-aaaaagcaggctAGATCTGCTAA AGAATGCTCTTCTGAT-3′) and NpLRX1-RNAi-AS (5′-agaaagctgggtCAATTGGTGCAACAGGTCTTGA-3′). Small letters indicate portions of the attB1 and attB2 sites, respectively, which were included in the primers for use in Gateway cloning. This fragment was introduced into the pDONR/zeo vector (Invitrogen) using BP Clonase (Invitrogen), followed by introduction into the RNAi vector pBCR86 (kindly supplied by Dr. Nuranaka of Plant Science Center, RIKEN) using LR Clonase (Invitrogen). The pBCR86 vector containing the *NpLRX1*-RNAi cassette was digested with *Avr*II and *Sac*I. The *Avr*II-*Sac*I 35S promoter-*NpLRX1*-RNAi cassette was purified and subcloned into a modified pBI101 in which the *NPTII* gene (neomycin phosphotransferase providing

kanamycin resistance) was replaced with the *HPT* gene (hygromycin phosphotransferase providing hygromycin resistance). These constructs were introduced into the *Agrobacterium tumefaciens* strain LBA4404 C58 Rif^r (pGV2260). Tobacco transformation and regeneration were performed using the leaf disk co-cultivation method (Horsch et al. 1985). To transform BY-2 and BY-GT 16 cells, we utilized the co-cultivation method and selected transformed cells on the modified LS medium containing the appropriate antibiotics.

Histochemical assay for GUS activity

To detect GUS activity, roots and leaves were harvested from each transformant and incubated in coloration buffer [100 mM phosphate pH 7.5 containing 2.5 mg of X-gluc (5-bromo-4-chloro-3-indolyl β-D-glucuronide cyclohexylamine) per 10 ml of buffer, 2.5 mM ferricyanide, and 2.5 mM ferrocyanide]. After a 30-min infiltration treatment, the GUS reaction was allowed to proceed for 4–12 h at 37°C. Samples were destained in 70% ethanol before analysis.

Anatomical analysis using leaf cross-sections

For anatomical analysis, leaves of about 10 mm in length were isolated from the control and *NpLRX1*-RNAi transgenic adventitious shoots, and used for preparing plastic sections as described (Iwai *et al*. 2002). The sections were stained with 0.5% toluidine blue, and the stained samples were observed under a light microscope (DMRB;Leica).

Results

Identification of NpLRX1 *by T-DNA tagging*

We produced the *nolac* mutant through T-DNA transformation of leaf disks of haploid *N. plumbaginifolia*, and isolated the mutant cell line *nolac-K4*. *nolac-K4* callus cells were loosely attached, and the morphology of the cell clusters was irregular. The color of the *nolac-K4* callus was pale green. Maintaining the cells of this line in culture was difficult, similar to a number of other *nolac* mutants.

To characterize the T-DNA insertion sites, the genomic DNA flanking the left T-DNA border in *nolac-K4* was amplified by thermal asymmetric interlaced PCR (TAIL-PCR). Four genes tagged with T-DNA were identified. One was identified as a retrotransposon, and the other two genes, encoding mitogenic oxidase and aspartate kinase, did not appear to be responsible for the *nolac-K4* mutation. In the fourth flanking DNA region, a gene was identified that shows 55% homology to the *Arabidopsis* extensin-like protein gene. Subsequent sequence analysis confirmed that this gene encodes a protein with an amino-terminal LRR domain and a carboxy-terminal extensin domain (Figure 1). Comparison with entries in the *Arabidopsis* DNA database revealed that the LRR domain of this gene is homologous to *Arabidopsis LRX* (LRR-EXTENSIN) genes, including the previously characterized *Arabidopsis AtLRX1* gene (Baumberger et al. 2001). Because of this structure, we named this gene *NpLRX1* (accession number AB273718).

The *nolac-K4* mutant contains a T-DNA insertion 150 nt upstream of the translation start site and 33 nt downstream of the TATA box in *NpLRX1*. The primary structure of the protein consists of a predicted signal peptide, an LRR domain of 260 amino acids, and a carboxy-terminal extensin-like domain of 305 amino acids (Figure 1). The LRR domain contains ten repeats of 22–24 residues approximately matching the plant extracytoplasmic LRR consensus sequence LxxLxxLxLxxNxLxGxIPxxLGx (Jones and Jones 1997). The LRR domain of *NpLRX1* shares 53% sequence identity with the amino-terminal domain of *AtLRX1*, and also shows strong similarity to ten other putative *Arabidopsis* proteins, each of which contains a conserved LRR domain and a variable extensin domain. In a phylogenetic analysis, the *NpLRX1* sequence falls into a clade that contains *Arabidopsis LRX* genes that are expressed in vegetative tissue (Baumberger et al. 2003) (Figure 2).

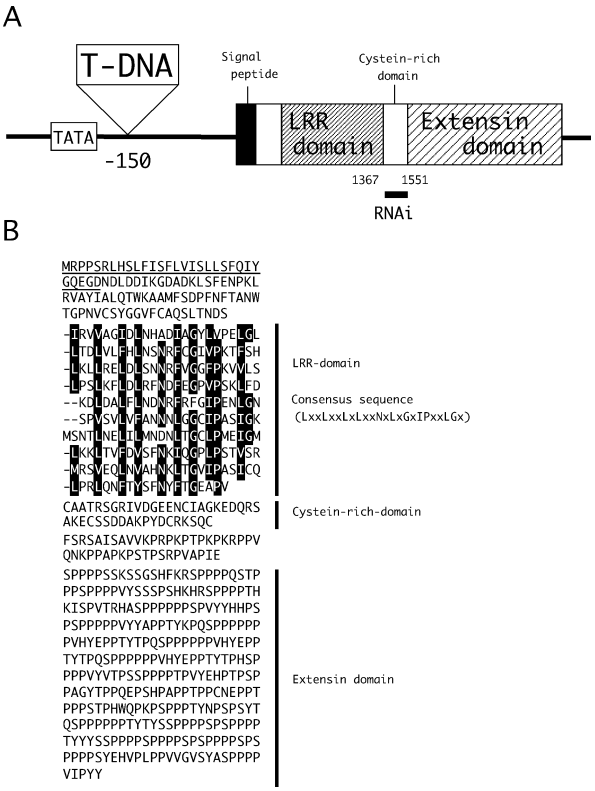

Figure 1. Structure of the *NpLRX1* gene. (A) Schematic representation of the *NpLRX1* gene. The black box indicates the predicted signal peptide. The shaded boxes indicate the positions of the LRR and extensin domains, respectively. The T-DNA insertion site is indicated by a triangle. The cysteine-rich domain specific to NpLRX1 between the LRR and extensin domains was used to design an RNAi construct and RT-PCR primers. (B) Deduced amino acid sequence of *NpLRX1* gene. The LRR region is aligned on the plant extracellular LRR consensus sequence (LxxLxxLxLxxNxLxGxIPxxLGx). The conserved amino acids are indicated in black boxes. The under line indicates the signal peptide.

NtPEX1 was the only tobacco *LRX* homolog to be reported previously (Wong 2001). To identify the tobacco *LRX* gene family, we searched the tobacco EST database using, as a query, the portion of the *NpLRX1* sequence containing the cysteine-rich domain between the LRR and extensin domains (Figure 1). The results of this survey suggested that genes homologous to *LRX1* exist in *N. tabacum* and *N. benthamiana*. We isolated a full-length gene by amplification from *N. tabacum genomic* DNA using PCR primers designed to hybridize to sequences in the highly homologous region. Subsequent sequence analysis confirmed that the homologous gene encodes an LRR-extensin protein that shares 93% sequence identity with *NpLRX1*. The *N. tabacum* gene was named *NtLRX1* (accession number AB273719).

Expression pattern of NpLRX1

To estimate the functions of *NpLRX1*, we analyzed its expression pattern by a semi-quantitative reverse transcription-PCR (RT-PCR) analysis using total RNAs prepared from different organs. *NpLRX1* mRNA accumulation was found in every tested organs. *NpLRX1* was expressed at a relatively low level in the flower bubs and the flowers (Figure 3A). Because of this expression pattern, *NpLRX1* can be classified as a vegetatively expressed LRX. The *NpLRX1* expression pattern is more diverse than those of the phylogenetically related *AtLRX1* to 6 genes. To assess the histochemical localization of *NpLRX1* gene expression, the promoter region from −1307 and −132 nt of *NpLRX1* (*pNpLRX1*) was fused to the GUS reporter gene and introduced into *N. plumbaginifolia* plants. *pNpLRX1::GUS* plants showed ubiquitous expression of *NpLRX1* in vegetative tissues, with stronger expression in lateral root primordia, root apical meristems, root steles, and leaf veins (Figure 3B).

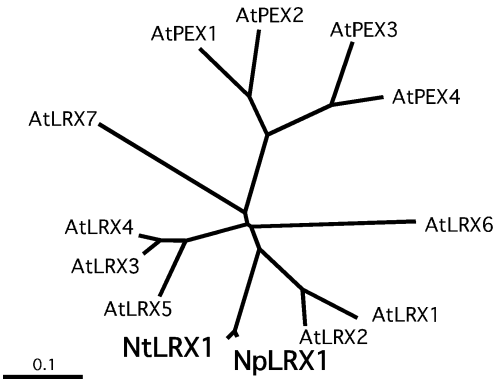

Figure 2. Phylogenetic relationships between NpLRX1, NtLRX1, and the *Arabidopsis* LRX family. The sequences of AtLRX1～7 and AtPEX1～4 were reported by Baumberger et al. (2003). The accession numbers of NpLRX1 and NtLRX1 are AB273718 and AB273719, respectively.

Transformation of tobacco leaf disks with NpLRX1-RNAi

To elucidate the function of *NpLRX1*, we generated *NpLRX1*-RNAi transgenic tobacco plants. To generate the RNAi construct, a 184-bp *NpLRX1*-specific region, encoding the cysteine-rich domain between the LRR and extensin domains, was used as an RNAi trigger region (Figure 1). The GENETYX alignment program did not reveal significant homology of this region with any *LRXs* or *PEXs*, except for *NtLRX*1 and its homologue in *N. benthamiana*. Transformation was performed with *N. plumbaginifolia* leaf disks, and transformants were selected in an adventitious-bud-inducing nutrient medium containing kanamycin. In the control experiment with *35S::GFP*, firm green callus formed on the leaf disks (Figure 4A). In contrast, transformation with *NpLRX1*-RNAi resulted in the formation of more than 60 white friable cell masses with weak cell adhesion on the leaf disks, with no formation at all of firm green callus (Figure 4B).

In *N. tabacum*, a number of green adventitious buds formed in the *35S::GFP* line, which showed normal regeneration (Figure 4C), but white adventitious buds

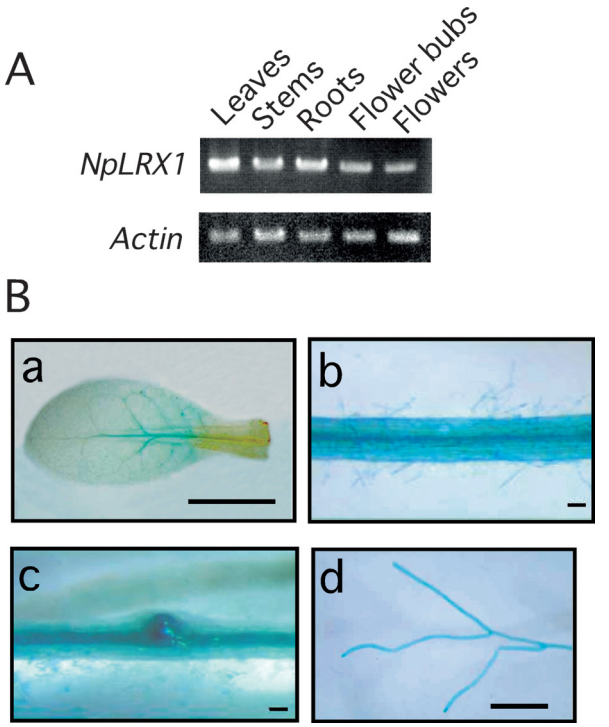

Figure 3. Expression profile of the *NpLRX1* gene. (A) *NpLRX1* transcript levels in various organs. The expression of the *NpLRX1* gene was monitored with a semi-quantitative RT-PCR. Actin was used as an internal control. The numbers of PCR cycles used for *NtLRX1* and actin were 30 and 25, respectively. (B) Tissues-specific expression of the *NpLRX1* gene. Tissues from transgenic *N. plumbaginifolia* containing the *NpLRX1* promoter fused to the *GUS* reporter gene were stained. *NpLRX1* expression was ubiquitously observed in every vegetative tissue, with stronger staining in the vascular bundles of leaves (a) and roots (b), the emerging lateral root (c) and root tip (d). Bars: 10 mm in a and d; 10 μm in b and c.

formed when *NpLRX1*-RNAi was expressed. In each *NpLRX1*-RNAi transformed bud, only one leaf formed and the shoot did not grow further, possibly because of a defect in the shoot meristem. (Figure 4D). Light microscopy of sections of the white leaves induced by *NpLRX1*-RNAi transformation revealed that the shapes of epidermal and mesophyll cells were aberrant, and large spaces were present between the cells (Figure 4E, F). Scanning electron microscopy of the leaf surface showed that the arrangement of epidermal cells was disturbed and the numbers of stomata were reduced (data not shown). RT-PCR analysis of the transformants showed reduced amounts of *NpLRX1* transcripts in *NpLRX1*-RNAi transformed leaves (Figure 4G).

Transformation of tobacco BY-2 cells with NpLRX1-RNAi

In tobacco BY-2 cells transformed with *NpLRX1*-RNAi, the proliferation of the cells was suppressed and the shapes of the cells were irregular, whereas *35S::GFP*-transformed cells grew in a cylindrical form (data not shown). The same result was obtained with BY-GT 16 cells into which GFP-fused tubulin had been introduced (Figure 5C). Observation of cortical microtubules using fluorescence microscopy revealed an irregular orientation of the cortical microtubules in *NpLRX1*-RNAi-transformed BY-GT 16 cells (Figure 5D), in contrast to the laterally parallel arrangement of these microtubules in control cells (Figure 5B).

Discussion

To confirm that *NpLRX1* is responsible for the *nolac-K4* mutation, it would have been ideal to show that the phenotype of *nolac-K4* can be complemented by *NpLRX1*. However, because the *nolac-K4* line perished before this could be accomplished, we generated a transformant in which the expression of endogenous *NpLRX1* or *NtLRX1* was suppressed by RNAi. The phenotype of this transformant showed characteristics similar to those of *nolac-K4* cells.

We first tried to generate *NpLRX1*-RNAi transgenic haploid *N. plumbaginifolia* plants. However, probably because of the lethality of the transformed cells, no *NpLRX1*-RNAi transgenic haploid *N. plumbaginifolia callus* was produced. Therefore, we generated *NpLRX1*-RNAi transgenic diploid *N. plumbaginifolia* cells. The friable white callus and weak cell adhesion of the transformant (Figure 4B) are similar to the characteristics of *nolac-K4* and are very common *in nolac* mutants. Because the transformed *N. plumbaginifolia* cells did not proliferate well, we attempted to produce *NpLRX1*-RNAi transgenic *N. tabacum* plants. Since searches of the tobacco BY-2 EST database suggested the presence of a gene homologous

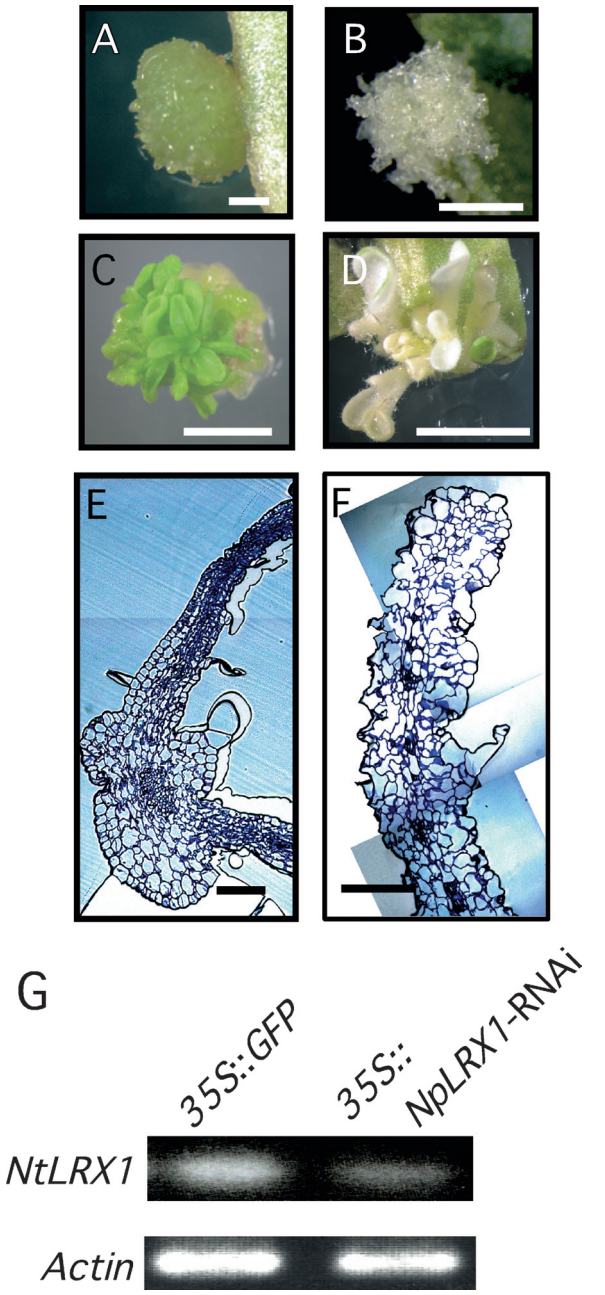

Figure 4. Phenotypes of *NpLRX1*-RNAi transgenic tobacco leaf disks. (A, B) Callus generated from *N. plumbaginifolia* leaf disks transformed with *35S::GFP* (A) or *35S::NpLRX1*-RNAi (B). (C, D) Adventitious buds generated from *N. tabacum* leaf disks transformed with *35S::GFP* (C) or *35S::NpLRX1*-RNAi (D). (E, F) Transverse sections of leaves of the adventitious buds in (C) and (D), respectively. The cell size and shape were abnormal and large gaps are evident between the cells in (F). (G) *NtLRX1* transcript levels in transgenic adventitious shoots expressing *35S::GFP* or *35S::NpLRX*-RNAi. The expression of the *NtLRX1* genes was monitored with semi-quantitative RT-PCR amplifying the cysteine-rich domain. Actin was used as an internal control. The numbers of PCR cycles used for *NtLRX1* and actin were 30 and 25, respectively.

to *LRX1* in *N. tabacum*, we isolated *NtLRX1* using PCR. Sequence analysis of *NtLRX1* revealed strong similarity with *NpLRX1*, including the *NpLRX1*-specific region (cysteine-rich domain) between the LRR and extensin

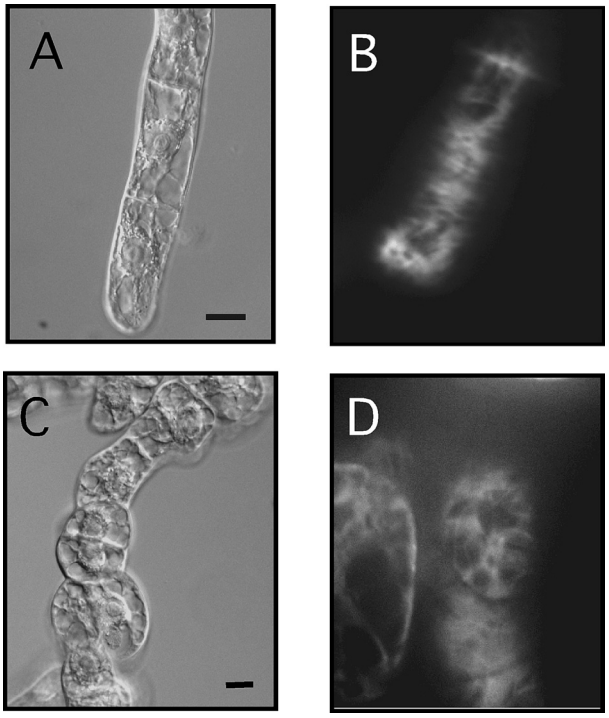

Figure 5. Phenotypes of *NpLRX1*-RNAi transgenic BY-2 cells. (A, C) Control BY-GT16 (A) and BY-GT16 cells transformed with *NpLRX1*-RNAi (C) observed using a microscope with Nomarski optics. (B, D) Bright fluorescence from GFP-tubulin in the cortical microtubules in control BY-GT 16 cells (B) and BY-GT 16 cells transformed with *NpLRX1*-RNAi (D) observed by fluorescence microscopy. Bars, 10 μm.

domains used as an RNAi trigger region. *NtLRX1* was successfully silenced using the *NpLRX1*-RNAi construct (Figure 4G), and the transformants showed a milder phenotype than the *N. plumbaginifolia* transformants. In particular, they formed adventitious buds with a single leaf and no shoot meristem. Microscopic observation of leaves suggested that the abnormal morphogenesis of the parenchyma and epidermal cells results in irregular cell arrangement in those tissues, with large gaps between the cells, in the process of adventitious shoot formation. A similar abnormal cell morphogenesis was also observed in *LRX1*-RNAi-transformed BY-2 cells, with a disturbance in the arrangement of cortical microtubules. These results and the ubiquitous expression of the gene in vegetative tissues (Figure 3) suggest that *Np* and *NtLRX1* have roles in cell morphogenesis in a wide variety of vegetative tissues.

The *Arabidopsis lrx1-null* mutant forms root hairs that swell, branch, or abort, suggesting that *AtLRX1* is involved in tip growth, a polarized type of growth (Baumberger et al. 2001). Another *Arabidopsis LRX* family, the *PEX*s, encodes proteins that localize in the walls of growing pollen tubes that are also elongating by means of tip growth (Rubinstein et al. 1995). Thus, the function of the *Arabidopsis* LRX proteins might be to control cell polarization or to locally regulate cell-wall expansion during tip growth. The other group of *AtLRXs* might function in the modification of localized cell-wall domains during the differentiation process; for instance, the preferential expression of *AtLRX3* and *AtLRX4* in vascular tissues might be related to a role of the LRX proteins in the maturation of xylem vessels (Baumberger et al., 2003). In contrast to the *Arabidopsis LRXs*, *NpLRX1* may be involved in more common processes that are required for cell morphogenesis in tobacco.

In root hairs, because polarization and growth orientation depend on both the microtubules and the Ca^{2+} gradient at the tip (Bibikova et al. 1997, 1999; Wymer et al. 1997), the root hair defects observed in the *lrx1* mutant have been postulated to be related to microtubules and membrane Ca^{2+} channels as possible direct or indirect targets for *LRX1* action (Baumberger et al. 2001). *NpLRX1*-RNAi BY-GT 16 cells show cell-shape abnormalities with irregular orientation of the cortical microtubules (Figure 5C, D). There might exist an integral cell membrane protein that communicates with cell-wall-localized *NpLRX1* to functionally associate with the cytoskeleton system.

In conclusion, the present study has demonstrated that *NpLRX1* plays an important role in maintaining the cell morphogenesis required for normal tissue development in tobacco cells, and further analysis of this factor should provide new knowledge of the control mechanisms of cell morphogenesis *via* apoplast-symplast communication.

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